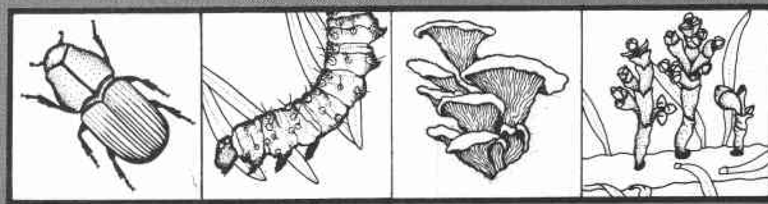
Forest Pest Management



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MORTALITY OF BAREROOT COLORADO BLUE SPRUCE SEEDLINGS MONTANA STATE NURSERY, MISSOULA

by

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INTRODUCTION

Bareroot conifer seedling production at the Montana State Nursery in Missoula has been difficult because of recurring losses from damping-off and root diseases (James, 1986d). As a result, seedling density has often been reduced and many surviving seedlings have been chlorotic and of generally poor quality. In an effort to improve bareroot seedling production, growers at the Nursery instituted a program of soil fumigation since it was evident that the major causes of problems were soil-borne pathogens, primarily species of *Fusarium* (James, 1986d). Therefore, during the late summer of 1986, production beds for the 1987 conifer seedling crop were fumigated with methylbromide/chloropicrin. This was the first instance of soil fumigation at the nursery.

Conifer seed were sown in the spring of 1987 and overall seedling emergence was good. However, mortality of Colorado blue spruce (*Picea pungens* Engelm.) became evident during July. Affected seedlings appeared to have become damaged during a short period of time with most mortality occurring within a few days. Groups of seedlings were affected (fig. 1). Many affected seedlings had constrictions on their stem just above the groundline and their roots were decayed. Investigations were conducted to determine causes of seedling mortality and evaluate efficacy of soil fumigation in reducing populations of pathogenic fungi.

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Figure 1.--Group mortality of 1-0 bareroot Colorado blue spruce seedlings due to heat injury at the Montana State Nursery, Missoula.

MATERIALS AND METHODS

Twenty seedlings, some of which had been recently killed and others in advanced stages of decline, were randomly collected from affected seedbeds. Selected seedlings were carefully excavated so as to include most of their taproots (most did not have well-developed lateral roots at the time of sampling). Seedling roots were thoroughly rinsed under running tap water for a few minutes to remove adhering soil particles. Roots were severed at the groundline, surface sterilized in 10 percent aqueous sodium hypochlorite for 2 minutes, and rinsed with distilled water. They were then aseptically dissected into 3-4 pieces, each about 5 mm in length. Root pieces were placed on a selective agar medium for *Fusarium* (Komada 1975). Plates were incubated at about 22 degrees C for 7-10 days under cool fluorescent light. Fungi emerging from root pieces were transferred to potato dextrose agar and identified. Fungi from the genus *Fusarium* were transferred to carnation leaf agar and identified using the taxonomic scheme of Nelson et al. (1983). Percentage of seedlings colonized with *Fusarium* and average root system colonization were calculated.

To evaluate the role of seed as a potential inoculum source of *Fusarium*, 150 seed were randomly selected from the seedlot sown in affected seedbeds. Seed were placed on a selective medium for *Fusarium* (Komada 1975) and plates incubated as described above. Percentage of sampled seed that was colonized with *Fusarium* was calculated.

Ten soil samples were collected from within affected seedbeds of Colorado blue spruce for assay of populations of two groups of potentially pathogenic fungi (*Fusarium* and *Pythium*). Five of the samples were collected near groups of diseased (symptomatic) seedlings and five adjacent to healthy-appearing (asymptomatic) seedlings. Each sample consisted of a core (23 mm in diameter) of soil taken to a depth of about 15 cm. Soil was placed in paper bags and transported to the laboratory for analysis.

Each soil sample was passed through an 8-mesh sieve to remove rocks and larger particles of organic material. A 5 g subsample of each soil sample was used to calculate oven-dry weight (used to provide a standard basis for comparison). For this determination, soil was dried at about 100 degrees C for 24 hours or until the weight became stabilized. For the *Fusarium* assay, 0.5 g of soil was weighed from each sample and mixed with 100 ml of 0.3 percent water agar. The mixture was then dispensed as l ml aliquots onto a selective medium for *Fusarium* (Komada 1975). Plates were incubated at about 22 degrees C under cool fluorescent light for 5 days. Number of *Fusarium* colonies per plate were determined and the colony-forming units per g of soil calculated. For the *Pythium* assay, 5.0 g of soil were mixed with 100 ml of 0.3 percent water agar. The mixture was then dispensed as l ml aliquots onto a selective medium for *Pythium* which consisted of V-8 juice agar amended with rose bengal, pimaricin, rifamycin, ampicillin, and pentachloronitrobenzene. Plates were incubated in the dark at about 24 degrees C for 3 days. Number of *Pythium* colonies per plate were determined on the basis of colony growth rate, growth within the agar, and uptake of the rose bengal dye. The number of colony-forming units per g of soil was calculated. All calculations of colony-forming units were made on the basis of soil oven-dry weight.

RESULTS AND DISCUSSION

Eighty-five percent of the seedlings sampled were infected with *Fusarium* (table 1). Percentage of root system colonization varied among the infected seedlings, but the average was about 50%. Results from soil assays for *Fusarium* and *Pythium* (table 2) indicated very low populations of both groups of fungi. No differences were apparent between samples collected near diseased seedlings and those collected adjacent to healthy (asymptomatic) seedlings. It appeared that soil fumigation successfully reduced amounts of *Fusarium* and *Pythium* to levels where they should not be problems.

Samples of spruce seed resulted in a *Fusarium* infection rate of 2.7 percent, well within expected normal rates (James 1985; James 1987a). Such low levels of infection would probably not result in significant amounts of seedling mortality, although some isolated damping-off might result.

The only species of *Fusarium* recovered from seed and seedlings was *F. oxysporum* Schlect. Although this species is a common pathogen of conifer seedlings (Bloomberg 1976; James 1986a), it can also infect seedlings without eliciting disease symptoms (James 1986c; James and Gilligan 1987; and James et al. 1987).

Although there was a high level of recovery of *F. oxysporum* from dead and dying seedlings, it did not appear that the fungus was responsible for the majority of the mortality. Most affected seedlings had prominent basal stem constrictions indicative of heat injury (James 1986b). Colonization of roots of heat injured seedlings by *F. oxysporum* may have occurred following injury.

It appeared that neither seed nor soil were major sources of *Fusarium* inoculum. Losses in nearby fumigated Douglas-fir seedbeds were attributed to infested seed (James, 1987b). However, the major source of *F. oxysporum* that colonized the roots of injured blue spruce seedlings was unknown.

To reduce future damage, seedlings should be misted during periods of expected high temperature, especially when they are young and succulent. The problem was probably not due to *Fusarium* root disease, and soil fumigation effectively reduced populations of pathogenic fungi to acceptable levels.

Table 1.--Isolation results from 1-0 bareroot Colorado blue spruce seedlings that were declining or had recently died at the Montana State Nursery, Missoula.

Seedling Number	Condition*	Infected with Fusarium	Percentage Root System Infected**
1	1	Yes	33.3
2	1	Yes	50.0
3	3	No	-
4	2	Yes	25.0
5	1	Yes	100.0
6	2	No	-
7	2	Yes	25.0
8	1	Yes	50.0
9	2	Yes	25.0
10	1	Yes	66.7
11	1	Yes	100.0
12	1	Yes	66.7
13	1	Yes	25.0
14	2	Yes	25.0
15	2	Yes	25.0
16	2	No	-
17	2	Yes	66.7
18	3	Yes	50.0
19	1	Yes	100.0
20	1	Yes	50.0

Percent Infected - 85.0
Average - 50.8

*Condition Classes:
1 = Brown (All foliage necrotic).
2 = About 1/2 of the foliage necrotic and about 1/2 chlorotic.
3 = Less than 1/2 of the foliage necrotic; greater than 1/2 chlorotic.

**Percentage of root pieces colonized by *Fusarium*.

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Table 2.--Results of soil assays for presence of *Fusarium* and *Pythium* at the Montana State Nursery, Missoula.

			Colony Forming Units/g	
Sample Number	**Location***	**Oven-dry Weight Factor**	*Fusarium*	*Pythium*
1	D	1.187	0	6
2	D	1.256	0	0
3	D	1.097	0	2
4	D	1.114	0	0
5	D	1.632	0	2
Average	-	-	0	2.0
6	ND	1.095	0	0
7	ND	1.115	0	2
8	ND	1.213	200	0
9	ND	1.407	200	8
10	ND	1.019	0	4
Average	-	-	80.	2.8
Total Average	-	-	40.	2.4

*D = Sample collected adjacent to diseased seedlings; ND = sample collected adjacent to healthy (asymptomatic) seedlings.

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